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MicroRNA 与转录因子相互作用对基因表达影响的研究

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题名页

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Effect of dynamic interaction between microRNA and transcription factor on gene expression

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摘要

MicroRNA (miRNA) 是内生的非编码 RNA，通常涉及动物和植物不同的生物过程。它们与转录因子和下游基因相互作用，形成复杂并且高度关联的调控网络。为了认识一些发挥重要基础调控作用的代表性模块，我们构建了一个包含 miRNA 的前馈环路模型，该模型包含转录因子调控 miRNA 和目标基因。根据 miRNA 和转录因子对基因表达不同的相互作用关系，四种可能的前馈环路结构与两类 gate 函数被引入。当转录因子的生成率是常数时，我们发现除了 Co1 环路外，其它模块中的目标基因水平都表现出脉冲行为。进一步，当系统输入信号为方波刺激时，我们观察到在 AND gate 条件下的 In2 环路中，目标基因在刺激出现时更鲁棒；当刺激去掉时，在 AND gate 条件下的 In1 环路中目标基因更稳定。同时，我们也研究了在方波刺激条件下，响应时间与 miRNA 最大活性速率之间的关系。我们发现在 OR gate 条件下的 Co1 环路中响应曲线是非单调的，这有可能帮助我们推断 miRNA 与启动子结合的机制。最后我们研究了重要参数对系统动力学响应的影响。我们发现所有的环路中稳态的目标基因水平对 miRNA 的初值变化不敏感。

关键词： 前馈环路；网络模块；动力学响应；miRNA；基因表达

英文摘要

MicroRNAs (miRNA) are endogenous non-coding RNAs which participate in diverse biological processes in animals and plants. They are known to join together with transcription factors and downstream gene, forming a complex and highly interconnected regulatory network. To recognize a few overrepresented motifs which are expected to perform important elementary regulatory functions, we constructed a computational model of miRNA-mediated feed-forward loops (FFLs) in which a transcription factor (TF) regulates a miRNA and target gene. Based on the different dynamic interactions between miRNA and TF on gene expression, four possible structural topologies of FFLs with two gate functions (AND gate and OR gate) are introduced. When the synthesis rate of TF is constant, we found that except Co1 loop, the dynamics of target gene in other motifs show pulse-like behavior. Moreover, when providing the system with simultaneous pulse, we observed that the target gene in In2 loop with AND gate is more robust in the presence of stimulus addition, and the target gene in In1 loop with AND gate is more stable after an off step of stimulus. Also, we studied the relationship between the response time and maximal activation velocity of miRNA when providing the system with simultaneous pulse. We found that the curve of response time shows non-monotonic behavior in Co1 loop with OR gate. This may help us to infer the mechanism of miRNA binding to the promoter region. At last we investigated the influence of important parameters on the dynamic response of system. We identified that the stationary levels of target gene in all loops were insensitive to the initial value of miRNA.

Keywords: Feed-forward loop; network motif; dynamical response; miRNA; gene expression

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第一章 Introduction

MicroRNAs (miRNA) [1, 2] are a class of endogenous small non-coding RNAs that bind to partially complementary sequences in target mRNAs, negatively regulating their protein production in higher eukaryotes, plants and animals [1, 3-5]. Many experimental studies have revealed that miRNAs can regulate various biological functions [6, 7], for instance, development and metabolisms [8]. Also, they have been demonstrated to be involved in many cellular signaling regulation processes, including apoptosis, proliferation and differentiation [9-11]. Moreover, a lot of biological and clinical experiments have shown that miRNAs are involved in the initiation and development of many diseases [12, 13], such as cancers [14] and HIV [15]. More and more attention has been focused on the molecular mechanisms related to miRNA and their functions [16].

The production of miRNA is regulated by certain transcription factors (TFs) that are also key regulators in gene expression. It has been demonstrated that miRNAs and TFs are often highly interacted in a dependent or independent manner [17]. Therefore, miRNA functions can be understood more clearly only in the context of regulatory interactions between TF and miRNA. Experimental data have demonstrated that gene regulatory networks are often constituted of some basic subcircuits involving feed-forward or feedback loops [18], which are often called as motif [19].

Feed-forward loops (FFLs) have been shown to be a major member of biological network motifs. Many theoretical works [20-22] and experimental studies [23] have been conducted to investigate their structure and functions within the context of gene expression regulation. These studies focused on FFLs at the transcriptional level, in which gene expression is controlled by two regulatory TFs. Moreover, certain miRNA-containing motifs are often embedded in a lot of gene regulatory networks (GRNs). It has been known that all miRNAs operate through a repressive action on target mRNA. However, considering the interaction between miRNA and TFs, the role of miRNA in gene regulatory network is not simply repressive. Therefore, the

investigation of the effect of interaction between TF and miRNA on gene expression is very important to help us understand the role of miRNAs in the GRN and disease.

Mathematical model is a powerful tool used to describe the biological systems and discriminate between different tentative mechanisms [24-36]. Several studies have examined the mechanisms of miRNA-containing motifs using mathematical models. Osella et al. [37] used a detailed analytical model and simulations to investigate the function of the miRNA-mediated FFL. Their analysis demonstrated that the incoherent version of such FFL motif can provide precision and stability to the overall gene expression program with an efficient noise control, given the existence of fluctuations in upstream regulators. Morozova et al. [38] developed a mathematical model containing nine known mechanisms of miRNA action and discriminated among different possible individual mechanisms based on the kinetic signatures. Duk et al. [39] analyzed three mathematical models, in which miRNA either represses translation of its target or promotes target mRNA degradation, or is not re-used, but degrades along with target mRNA. They showed that different mechanisms of miRNA action lead to a variety of types of dynamical behavior of feed-forward loops. However, none of previous studies examined the effects of dependence (AND gate) or independence (OR gate) between miRNA and TFs on gene expression.

In this report, we developed a mathematical model to quantitatively analyze the dynamics of miRNA-containing FFLs and investigate the interaction between miRNA and TF on gene expression. We examined four FFLs, in which each contains AND gate or OR gate. We analyzed the different dynamical behaviors between AND gate and OR gate for each of these four FFLs. Our results showed that different mechanisms with respect to AND or OR gate might produce distinct dynamics of the GRN. In addition, we examined the relationship between response time of gene expression and certain parameters in the model. Finally we investigated the influence of important parameters on the response of system. Our study advances our quantitative understanding on the dynamic interaction between TF and miRNA,

particularly, with AND or OR gate in the GRN, and provides some implications on the miRNA mediated dieses.

第二章 Results

2.1 Mathematical model of FFLs

Fig. 1 illustrates the general structure of FFLs in miRNA-mediated gene transcription network, similar as that in Refs. [24-27]. The upstream transcription factor (TF) regulates the target gene via two parallel pathways: directly, and by interaction with miRNA, which also regulates the target gene. Therefore regulatory interactions in FFL create four possible structural topologies (Fig. 1). Two of these configurations are named "coherent": the sign of the direct regulation path from TF to gene is the same as the overall sign of the indirect regulation path from TF via miRNA to gene. The other two structures are termed "incoherent": the sign of the direct regulation path is opposite to that of indirect path. We specify these configurations as the type 1 or 2 coherent FFLs, and the type 1 or 2 incoherent FFLs, respectively. The biological network motif under investigation is described by 3 variables, the concentrations of transcription factor (X), miRNA (Y) and target gene (Z). The dynamical behavior of the FFLs is governed by the following equations,

$$\frac{dX}{dt} = k_1 - d_1 X \tag{1}$$

$$\frac{dY}{dt} = v_2 f(X, k_{12}) - d_2 Y \tag{2}$$

$$\frac{dZ}{dt} = v_3 g(X, k_{13}; Y, k_{23}) - d_3 Z \tag{3}$$

The regulation function for an activator is $f(\mathrm{u}, \mathrm{k}_{ij}) = (\mathrm{u}/\mathrm{k}_{ij})^n / (1 + (\mathrm{u}/\mathrm{k}_{ij})^n)$, and for a repressor is $f(\mathrm{u}, \mathrm{k}_{ij}) = 1/(1 + (\mathrm{u}/\mathrm{k}_{ij})^n)$, similar as that we used before in Refs. [40, 41]. $g(X, k_{13}; Y, k_{23})$ is the gate function, the mechanisms underlying miRNA mediated repression are not clear so far, and for this reason we consider the gate function has two forms. The gate function for an AND gate is $g(X, k_{13}; Y, k_{23}) = f(X, k_{13}) * f(Y, k_{23})$, while for an OR gate we have $g(X, k_{13}; Y, k_{23}) = f(X, k_{13}) + f(Y, k_{23})$. For more details about the values of parameters and initial concentrations we use, see Tables 1 and 2.

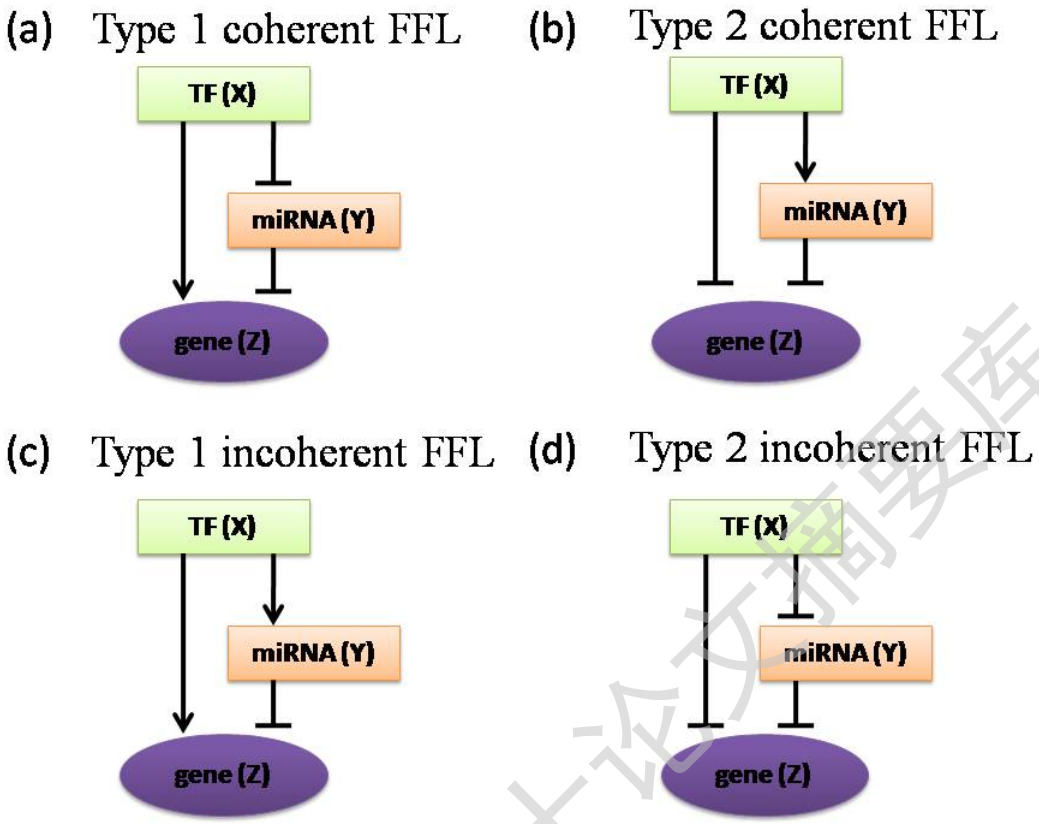

Fig. 1: The coherent and incoherent feed-forward loops. Arrows mean activation, the turned over T-bars indicate repression. (a) Type 1 coherent FFL, TF activates target gene and represses miRNA synthesis. (b) Type 2 coherent FFL, TF represses target gene and activates miRNA synthesis. (c) Type 1 incoherent FFL, TF activates both target gene and miRNA synthesis. (d) Type 2 incoherent FFL, TF represses both target gene and miRNA synthesis.

Table 1: **The values of parameters in the mathematical model**.

Para No.	Symbol	Value	Description
1	d_1	0.2	degradation rate of TF
2	v_2	1.0	maximal activation velocity of miRNA by TF
3	d_2	0.2	degradation rate of miRNA
4	v_3	1.0	maximal activation velocity of target gene by TF and miRNA
5	d_3	0.2	degradation rate of target gene
6	k_{12}	1.0	Michaelis constant of of miRNA by TF

7	k_{13}	1.0	Michaelis constant of target gene by TF
8	k_{23}	1.0	Michaelis constant of target gene by miRNA
9	n	2	Hill coefficient

Table2: Initial values of the mathematical model.

Para No.	Symbol	Value	Description
1	X	0	Initial value of TF
2	Y	0	Initial value of miRNA
3	Z	0	Initial value of target gene

2.2 Comparative analysis of FFLs' temporal behavior under different gate functions

We shall use for brevity the following abbreviations for the FFL identification: Co1 will mean the type 1 coherent FFL, Co2 – the type 2 coherent FFL, In1 – the type 1 incoherent FFL and In 2 – the type 2 incoherent FFL, respectively.

Fig. 2 shows the time courses of Z in various FFLs with different gate functions when k_1 is constant number. Here k_1 represents the basal synthesis rate of TF. The dynamics of target gene in Co1 loop has a form of increasing function, and then tends to a constant vale (Fig. 2a). The target gene profiles in Co2, In1 and In2 loops show pulse-like behavior due to repression mediated by miRNA (Fig. 2 b,c,d). At the steady state, the concentrations of target gene in all the loops with AND gate are much lower than those with OR gate function. It is easy to understand this, because OR gate function makes the synthesis rate bigger than that of AND gate.

Living cells constantly have to respond to a changing environment. To understand how cells deal with a fluctuating environment, we need to know how cells transduce time varying signals. Next we consider the effect of providing the system with simultaneous pulse, a biological scenario which corresponds to continued exposure to environmental stimuli within a certain time range. Accordingly, we set k_1 to be a piecewise constant function

$$k_1 = \begin{cases} 1 & 50 \le t \le 100 \\ 0 & otherwise \end{cases}$$

Fig. 3 shows the variations in the response of the output in the motifs. We first compare the kinetics of Z in Co1 and In1 loops (Fig. 3 a,c). When k_1 turns on, we find out only the steady states of Z in Co1 and In1 loops with both gate functions rise up due to the direct activation of Z by TF (Fig. 3 a,c). But in In1 loop, Z first rises slightly, and then falls down because TF inhibits Z by promoting miRNA. When k_1 turns off, both the concentrations of Z in Co1 and In1 loops decrease, but Z in In1 loop with OR gate eventually grows again to the stationary level. We then compare the kinetics of Z in Co2 and In2 loops (Fig. 3 b,d), we observe that the concentration of Z in Co2 loop decreases as k_1 turns on and increases as k_1 turns off (Fig. 3(b)). But Z in In2 loop with OR gate rises up again to the steady state level after Z falls down, as k_1 turns on (Fig. 3(d)). While Z in In2 loop with AND gate just slightly decreases when k_1 changes to 1. Both Z in In2 loop with two types of gate functions show pulse-like behavior after k_1 turns to 0, however, the amplitude of Z in In2 loop with OR gate is much smaller than that with AND gate. From the subfigures in Fig. 3, we can find that Z in In2 loop with AND gate is more robust in the presence of k_1 addition, and Z in In1 loop with AND gate is more stable after an off step of k_1.

The response time is a measure of the time it takes a gene product to reach its physiologically determined steady state level. The speed of the response is characterized by the response time, that it takes Z to reach half of its steady state level. Here v_2 is the maximal activation velocity of miRNA by TF. In Fig. 4, we study the relationship between the response time and v_2 in Co1 loop with both gate regulations when providing the system with simultaneous pulse. We can observe that the response time has a form of increasing function as v_2 turns bigger in Co1 loop with AND gate, which means the system responses more slowly as v_2 increases. This is easy to understand, larger v_2 induces more miRNA generation which further represses target gene synthesis, so the response time turns slowly. But for the case in Co1 loop with OR gate, the response time shows non-monotonic behavior, which first climbs and then damps as further increasing v_2. This indicates that there exists a value of v_2 such

that the system responses most slowly. To understand this, we need to refer to OR gate function we use. It is a non-monotonic function as v_2 increases, so the form of function decides the speed of the response of the system. Our result here might be useful to infer the mechanism of miRNA binding to the promoter region, whether or not the TF and miRNA compete for binding to the target gene. Also, we obtain that the response of gene expression in Co1 loop with OR gate is faster than that in Co1 loop with AND gate during the period of v_2 changing.

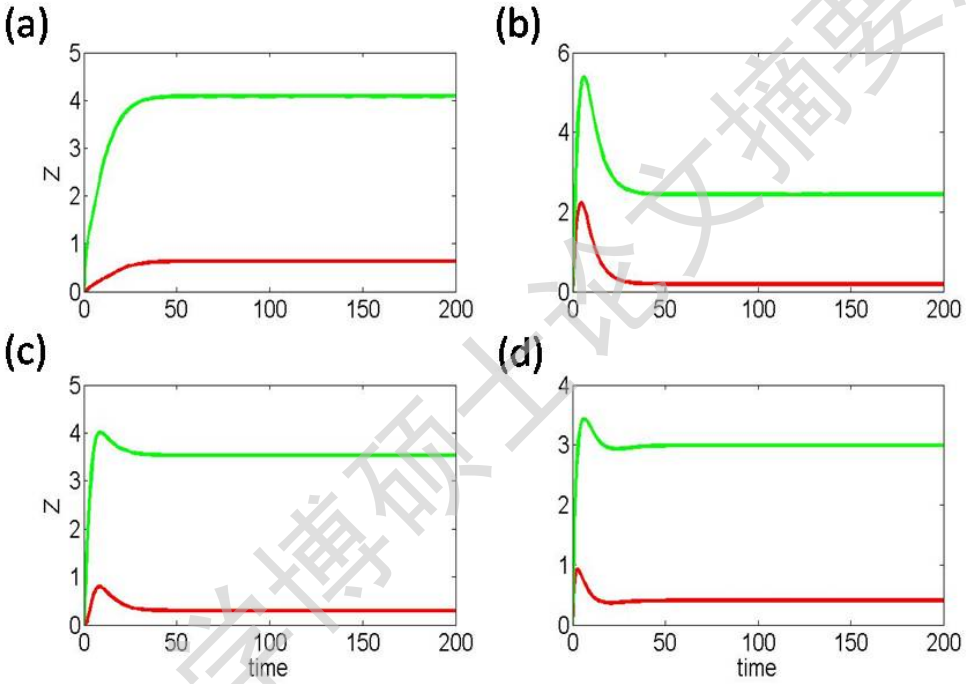

Fig. 2: The time evolutions of Z in various FFLs with different gate functions when k_1 is constant input. Type 1-2 coherent FFLs are shown in (a)-(b), while type 1-2 incoherent FFLs are given in (c)-(d). The red line corresponds to AND gate function, and the green line represents OR gate function. Here we fix $k_1 = 0.25$.

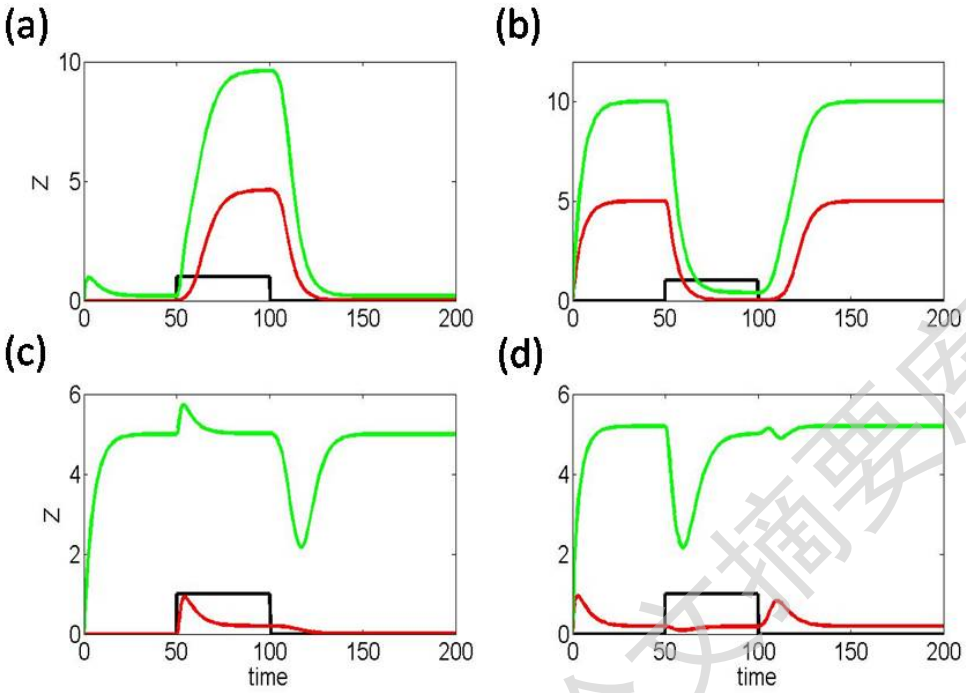

Fig. 3: The time evolutions of Z in various FFLs with different gate functions in response to on and off steps of k_1. Type 1-2 coherent FFLs are shown in (a)-(b), while type 1-2 incoherent FFLs are given in (c)-(d). The red line corresponds to AND gate function, and the green line represents OR gate function. k_1 is set to 1 during the time between 50 and 100, and 0 in other time range (the black line).

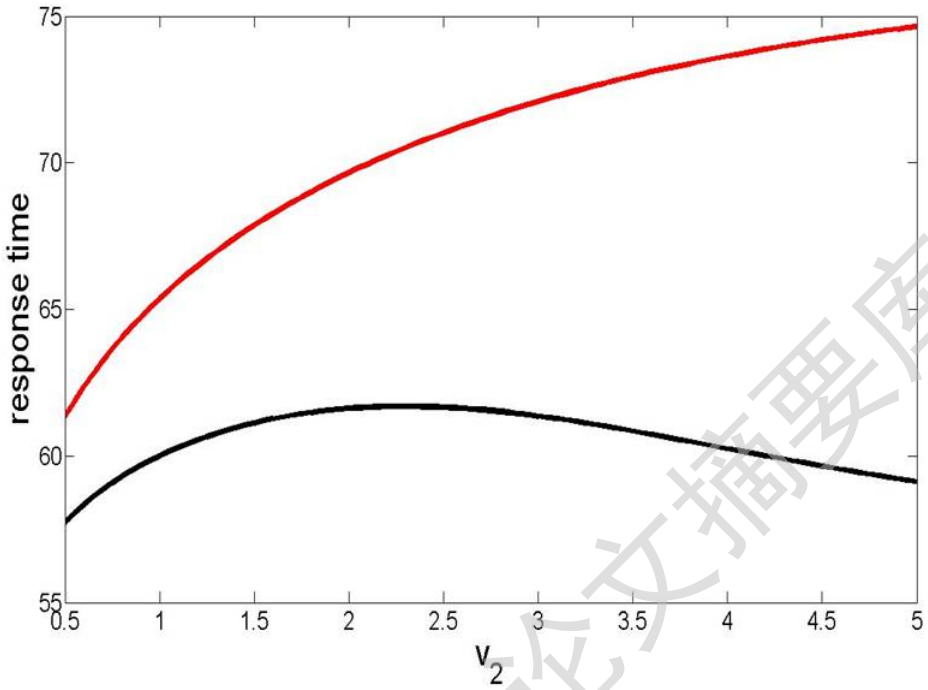

Fig. 4: The response time is plotted against the variation of v2 in Co1 loop with different gate regulations. The red line corresponds to AND gate function, and the black line represents OR gate function. k_1 is set to 1 during the time between 50 and 100, and 0 in other time range.

2.3 **Variations of parameters on the response of system**

It is known that the model coefficients might affect the dynamical behavior of FFLs. Therefore, we further examine how the changes in parameters affect the temporal behavior of the target gene. We investigate the effect of changes in v_2, d_2, k_1 and d_1 on the dynamical behavior of Z.

Fig. 5 shows the time course of Z in various FFLs with different gate functions in response to variation of v_2. We choose three typical values of v_2: the original value, 10-fold and 0.1-fold of v_2. We find bigger v_2 induces less expression of target gene when Z reaches the steady state. We can understand this from the interaction relationship in Fig. 1. Larger v_2 results in more miRNA generation which further represses target gene synthesis, so at last less target gene was observed. Parameter d_2